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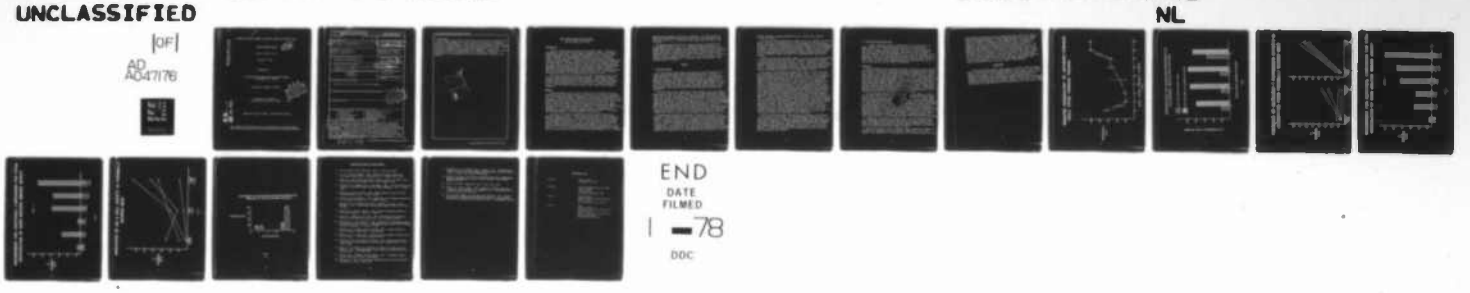

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STUDIES OF ALTERED RESPONSE TO INFECTION INDUCED BY THERMAL INJURY

ANNUAL PROGRESS REPORT

Carol L. Miller, Ph.D.

October 1, 1977

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Sepsis is a major cause of mortality after thermal injury. Experiments described ~~in this annual report~~ are designed to determine if thermal injury reduces host defenses to infection and to characterize the burn induced immune abberation(s). Specifically data are presented which investigate (1) that thermal injury directly damages leukocyte function, (2) the precise kinetics of the appearance of the burn-induced injury, (3) the leukocyte subpopulation (A, T, or B cell) that is appected by thermal injury, and (4) the mechanism by which thermal injury reduces immune leukocyte functions. (cont. on reversed side)

abberation → aberration

appected → affected

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20. continued.

An intrinsic immune defect resulting from thermal injury was demonstrated. Days 5 to 7 after thermal insult were identified as the time of maximally reduction in immunocompetence. Bone marrow derived lymphocytes' differentiation to specific antibody forming cells was not prevented by thermal injury. However, both normal Accessory cell and normal thymus dereived cell function in generation of specific antibody responses was abberant. Inappropriate activation of suppressive cells was demonstrated in our burned murine system and suggested as a causal mechanism for loss of host defenses to infection after burns.

ARMY CONTRACT ANNUAL PROGRESS REPORT

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Introduction

Sepsis is a major cause of mortality after thermal injury. Infectious complications after thermal insult appear to largely result from burn-induced, immunological aberrations. Thermal injury mediated loss of host resistance through depression of immunocompetence could have intrinsic or extrinsic causes. Thermal injury could actually compromise the ability of leukocytes to mount a *de novo* response. Alternatively, increased hydrocortisone, unbalancing of electrolytes, etc. could be affecting a whole variety of biological functions only one of which is the immune system. Direct burn induced immune dysfunction can result from aberrations in any of the three general types of immunocompetent leukocytes which cooperatively mediate the generation of immune responses. These three leukocyte subpopulations are the antigen specific bone marrow derived (B) cell, the antigen specific thymus derived (T) cell, and a third extremely heterogenous population of leukocytes termed accessory (A) cells.

This report describes the results of this year's experiments examining the effects of acute thermal injury on immunocompetence. Specifically, data are presented which investigate 1) that thermal injury directly damages leukocyte function, 2) the precise kinetics of the appearance of the burn-induced injury, 3) the leukocyte subpopulation (A, T or B cell) that is affected by thermal injury, and 4) the mechanism by which thermal injury reduces immune leukocyte functions.

Methods

In each experiment, BDF or C_{57} Bl/6 inbred mice of 16-18 weeks of age are obtained from the Diablo Mouse Colony at the University of California at Berkeley. Under light metofane anesthesia, littermate mice are shaven and then divided in two groups. One group receives a 10-20 % scald burn with 95° C water for 5 seconds (experimental mice) while the second group is not burned (sham-control mice). At specific times after injury, 2-4 mice from each group are sacrificed. These animals'spleens are removed, teased into single cell suspensions and cultured *in vitro* with sheep erythrocytes using a modification of the Mishell-Dutton culture technique (1). We monitor thermal injury effects on immunocompetence by measuring the formation of specific antibody forming cells (AFC). This system facilitates detection of cell immunoregulatory interactions.

"A" and/or T leukocytes are depleted or isolated from the burned mice. Purified, syngeneic, normal or control leukocytes are added to these depleted thermally injured populations. In this manner, normal A or T cells are supplied to the immunodepressed burned mice's cells. These experiments examine whether supplying normal, functional A or T cells restore the ability of the thermally injured leukocyte population to generate normal numbers of specific AFC. "A" cells are depleted from leukocyte populations according to the method of Ly and Mishell (2). T cells are lytically removed from leukocyte populations by treatment of the splenocytes with anti-T cells antisera and complement (3). Leukocyte

populations are depleted of B cells by irradiation. "B" cells can also be depleted from splenocyte populations by passing the cell preparations over nylon wool columns (4). These nylon wool columns remove A cells, as well as B cells.

The in vitro generation of AFC is assayed using the slide modification of the Hemolytic plaque assay (5). Leukocyte recovery from cultures is determined by counting a sample of the harvested, cultured cells on a Coulter Counter (Model ZH). The number of AFC are calculated for each pool of duplicate cultures by performing quadruplicate slide assays. Data are corrected for background plaques and expressed as AFC/10^6 recovered spleen cells. Allogeneic condition media is produced as described (6). In order to augment A cell function, 2-mercaptoethanol (2ME) is sometimes added to cultures at a final concentration of 5×10^{-5} M.

RESULTS

A. Burn Mouse Model

As a first step in these studies, the kinetics of the development of burn injury induced immunodepression were precisely established. The maximal immune depression in animals receiving a 10% scald burn occurs at days 5,6,7 and 8 after injury. These data are illustrated in Fig. I. Once this maximal depression period was determined, experiments analyzing the mechanism and leukocyte subpopulations involved in this burn induced immune depression could be focused on animals who were 5, 6, and 7 days post thermal insult. In this manner, we were able to reduce the number of animals required overall, while simultaneously increasing the number of animals examined in each individual experiment.

There have been several suggestions in the literature (7,8) that thermal injury causes a defect in T cell function. Most of these experiments examined PHA or MLR activity. Consequently, the defect described could actually involve either the T or the A cell population. This laboratory, as well as others, have shown that cooperative-interaction between T and A cells is required to generate either a human mitogen or a human MLR response (9,10). Our original experiments suggested that B cells from burned mice had normal reactivity in the AFC response. Media containing immunologically active factors produced by T and A cells (Allogeneic Conditioned Media) were cultured with B cells from thermally injured mice. In the presence of these normal A and T factors these B cells were able to generate as many AFC as the B cells from sham injured animals. Therefore, thermal injury does not result in a B cell defect in our model (See Fig. II).

Experiments were devised to determine if T cells from burned mice were defective in their cooperation with A and B cells during induction of an immune response. A purified normal murine T cell population was isolated as follows. First, the leukocytes were passed over a Sephadex G-10 column, depleting the A cells. Then the A depleted cells were passed over nylon wool columns depleting the B cells. The resulting A and B cell depleted population was 95-99% T lymphocytes. These normal purified T cells were co-cultured with cells from syngeneic thermally injured mice. The leukocytes from the burned mice were either untreated or T depleted prior to mixing them with the purified normal T cells.

The AFC responses in these experimental mice cell cultures were compared to those of controls.

The results of these experiments are shown in Fig. III. Addition of purified normal T cells to an immunodepressed A, T and B leukocyte population did not increase the number of AFC which these cells could generate. In two experiments, the number of specific AFC generated was slightly increased and in two experiments decreased. There was no statistically significant increase in the AFC response in these four experiments which examined 12 animals. However, when the T cells were first depleted from the burned animals' leukocyte population and normal T cells subsequently added, the numbers of AFC generated were significantly restored.

The data indicate that the burned animals' T cells are not just dysfunctional. If thermal injury had only impaired T cell function, then addition of normal T cells should have restored the response. In fact, T depletion of the burn leukocyte population was required before the normal T cells could supply T helper function. These data imply that a suppressor T cell was generated by thermal injury. Only after this suppressive T cell was depleted from the population (by anti-T antisera) could normal T cells supply helper function.

Even after T cell depletion and readdition of normal T cells, the burned mice's leukocytes did not generate normal numbers of AFC. This lack of total restoration could have any or all of the following causes: 1) the T suppressors were not completely removed by the anti-T antisera, 2) there are additional suppressor cells present that are not T cells, 3) there is an added A cell defect as well as the T cell defect in these animals, 4) the T depleted cells cannot be completely restored in our system. These four possibilities are difficult to distinguish experimentally. We have compared the number of AFC generated by burn depressed leukocytes to those generated by normal leukocytes under the following conditions: both populations untreated, both populations co-cultured with Allogeneic Conditioned Media (Allo CM) which contains A and T cell replacing factors, both populations cocultured with additional normal T cells, both populations T depleted and cocultured with Allo CM and additional syngeneic T cells. Results from two of these experiments are shown in Figs. IV and V. "T" depleted leukocytes from thermally injured mice were totally restorable when Allo CM and normal syngeneic T cells were added. These data eliminate the possibility that T depleted cells cannot be totally restored in our system. They do not, however, discriminate between the other three possibilities suggested for lack of total restoration by addition of normal T cells alone. The additional T cell factors supplied in the Allo CM could be overcoming residual suppressor T activity remaining after depletion. "A" cell factors in the Allo CM could be overcoming either the suppressive activity of A cell inhibitors or restoring a dysfunctional A cell population.

Some preliminary experiments have been done to determine if there is an A cell as well as T cell defect. In these experiments, syngeneic purified A cells from normal mice are added back to the leukocytes from burned mice. The burned mice's leukocytes are either A depleted or untreated. Data from these experiments, shown in Fig. VI, imply that only slight restoration is achieved by A depletion and subsequent readdition of normal A cells. If the A and T defects were independent, we would expect to see greater restoration than is achieved. The data are consistant with the hypothesis that the T suppressor may be depressing A cell function.

B. Nutritional Deprivation Model

Protein deprivation causes an immune defect in our experimental rat model. However, our experimental system focuses only on the protein production by B cells. Protein depletion results in a striking reduction in the B cells' protein synthesis capacity. This defect is totally different from the thermally induced immune defect where the B cells are functional. This rat model does not allow examination of A and T cell function after protein depletion. However, our model has proven extremely valuable in analyzing the effectiveness of various total parental nutritional solutions (TPN). As can be seen in Fig. VII, TPN restores immunocompetence to protein deprived rats at a significantly greater rate than does oral refeeding.

Discussion

The data obtained in this laboratory establish that thermal injury directly interferes with the ability of leukocytes to generate a de novo antibody forming cell response. The maximal immunodepressive effect occurred at days 5, 6, and 7 after thermal insult. Data from several laboratories suggest that burn injuries affect more than one leukocyte subpopulation (7, 11, 12). Our data suggest that thermal insult causes aberrations in both T and A cell normal immune function. The B cell function appears unaffected. These data are compatible with other investigators' work showing a dramatic reduction in alveolar macrophage function (A cell function) following thermal insult (8, 13). It appears highly likely therefore that an A cell dysfunction results from thermal injury. Although we had difficulty definitively establishing an A cell dysfunction, our data suggest defective A cell function. The simultaneous presence of some types of T cell aberrations would interfere with detection of A cell defects.

Some experimental evidence suggests that thermal injury induces a T cell as well as A cell defect (14, 15). Several authors have postulated that thermal injury results in an increase in T suppressor cells (16, 17). However, there was no strong experimental support for this hypothesis. Our data are probably the best collaborative evidence yet available for demonstration of the activation of T suppressors after burn injuries. In our experiments, we showed that addition of normal syngeneic T cells to a leukocyte population from burned mice did not restore the immune function. Only after the burned mice's own T lymphocytes were removed by anti-T antisera, could normal T lymphocytes restore immune activity. These data establish a T cell defect since addition of normal T cells could restore the specific AFC response. Additionally, it demonstrates that the burn animals had suppressive T cells in their leukocyte populations because only prior removal of these T cells allowed the normal T cell function to be expressed.

Interestingly, the addition of normal T cells, even to T depleted burned mice's leukocytes, gave only partial restoration of the normal numbers of AFC. These data suggested that either more than one type of suppressor was present and/or that there was some type of interactive mechanism between the suppressor T cell we identified and the A cell defect we found.

There are two types of murine T suppressor cells, specific and nonspecific. The specific T suppressor has as its target a T helper cell (18). Such specific T cell suppression is restricted only to the inducing antigen (18, 19).

The nonspecific T suppressor has as its target the A cell (20, 21). In our system we are measuring the burn induced immunodepression of the anti-sheep red blood cell (SRBC) AFC response. It seems highly unlikely that thermal injury induces only specific T suppressors for the SRBC response. A nonspecific T suppressor is more likely induced by thermal insult. Such a nonspecific T suppressor would depress all subsequent responses (SRBC, bacteria, etc.). It therefore seems reasonable to assume that we may be detecting a murine T cell suppressor which activates A cell inhibitors. This would explain why we had difficulty clearly identifying the A cell defect after thermal insult. Further experiments are now in progress to test this possibility. This demonstration of a burn induced T suppressor's interaction with A cells would be exciting. It would be the first demonstration of this suppressor T cell pathway resulting from a natural pathology. It will also provide needed information of the leukocyte population(s) which should be treated in immunotherapy.

CONCLUSIONS

These data show that thermal injury results in a direct, intrinsic immune defect. The peak of the reduced immunocompetence occurs at day 5, 6 and 7 after thermal insult. Both A and T leukocyte functions in generation of specific AFC responses are aberrant. The burned mouse has a greatly reduced ability to recognize an invading bacteria and produce a de novo antibody response. "T" suppressor cells are activated after thermal insult. The data also suggest these T suppressors have some causative interaction with the A cell defect. It therefore appears that immunotherapy would be of considerable value to the burn victim.

REDUCED GENERATION OF ANTIBODY FORMING CELLS AFTER THERMAL TRAUMA

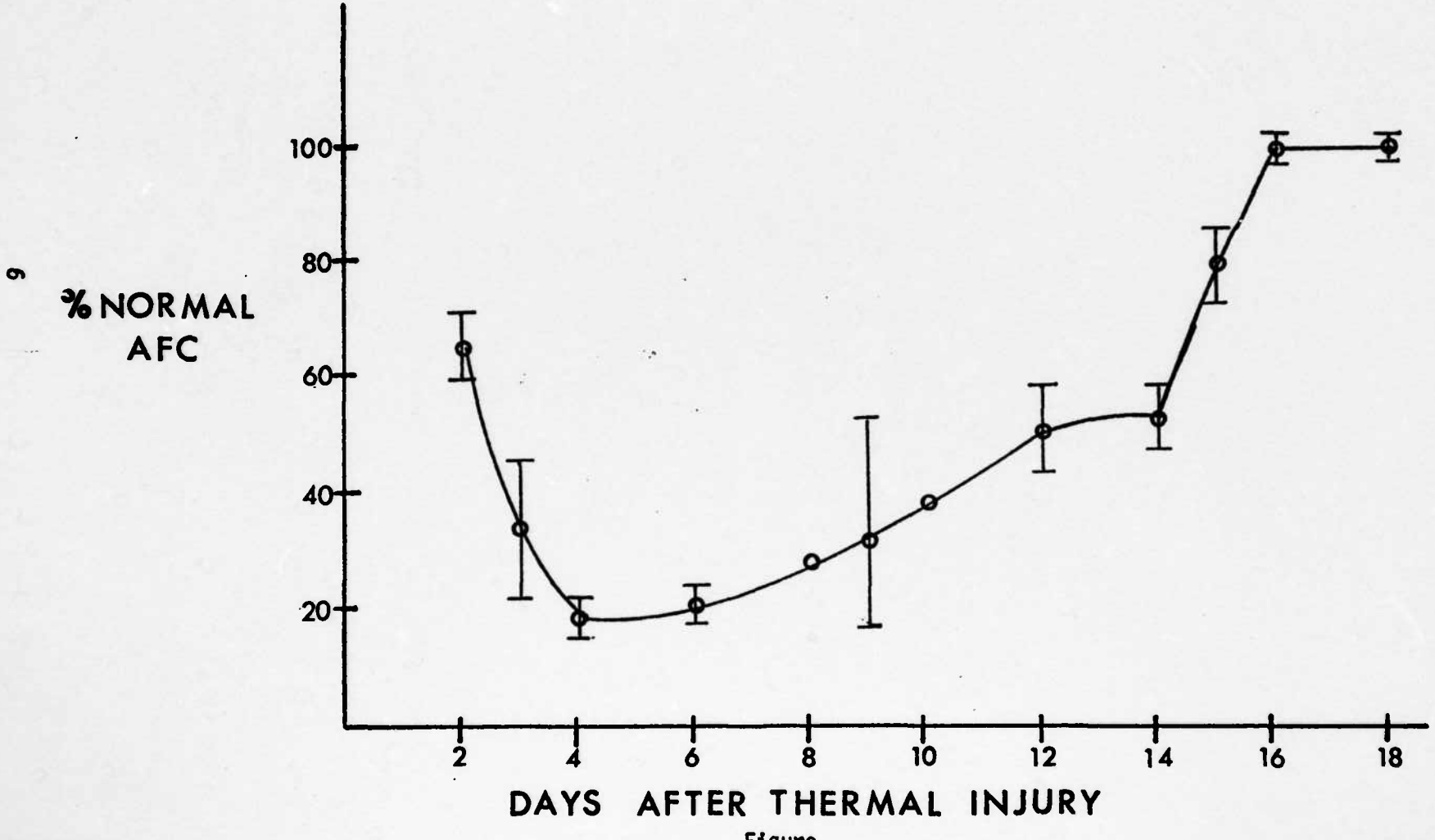


Figure I

RESTORATION OF IMMUNOCOMPETENCE BY ALLOGENEIC LEUCOCYTE FACTORS

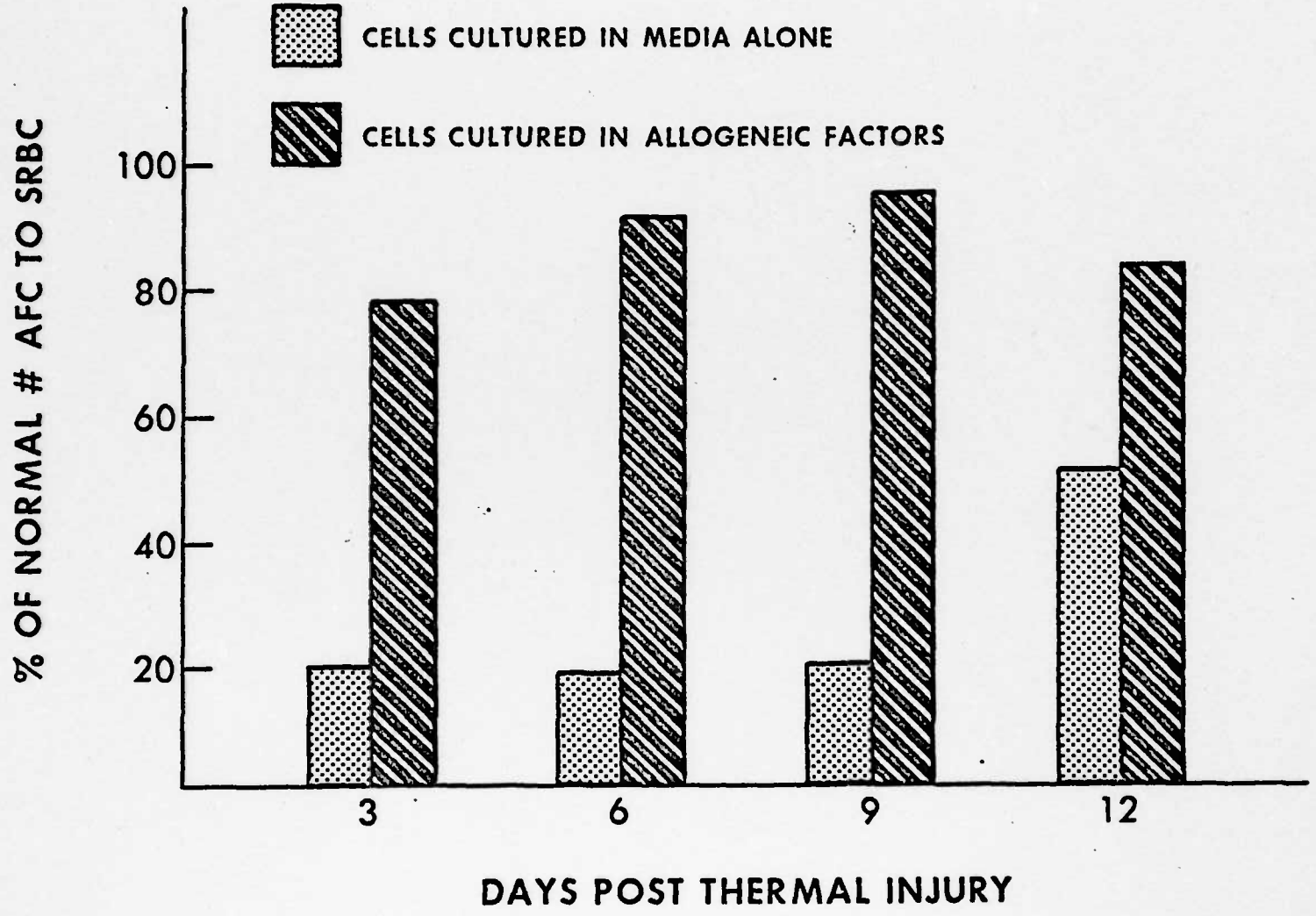
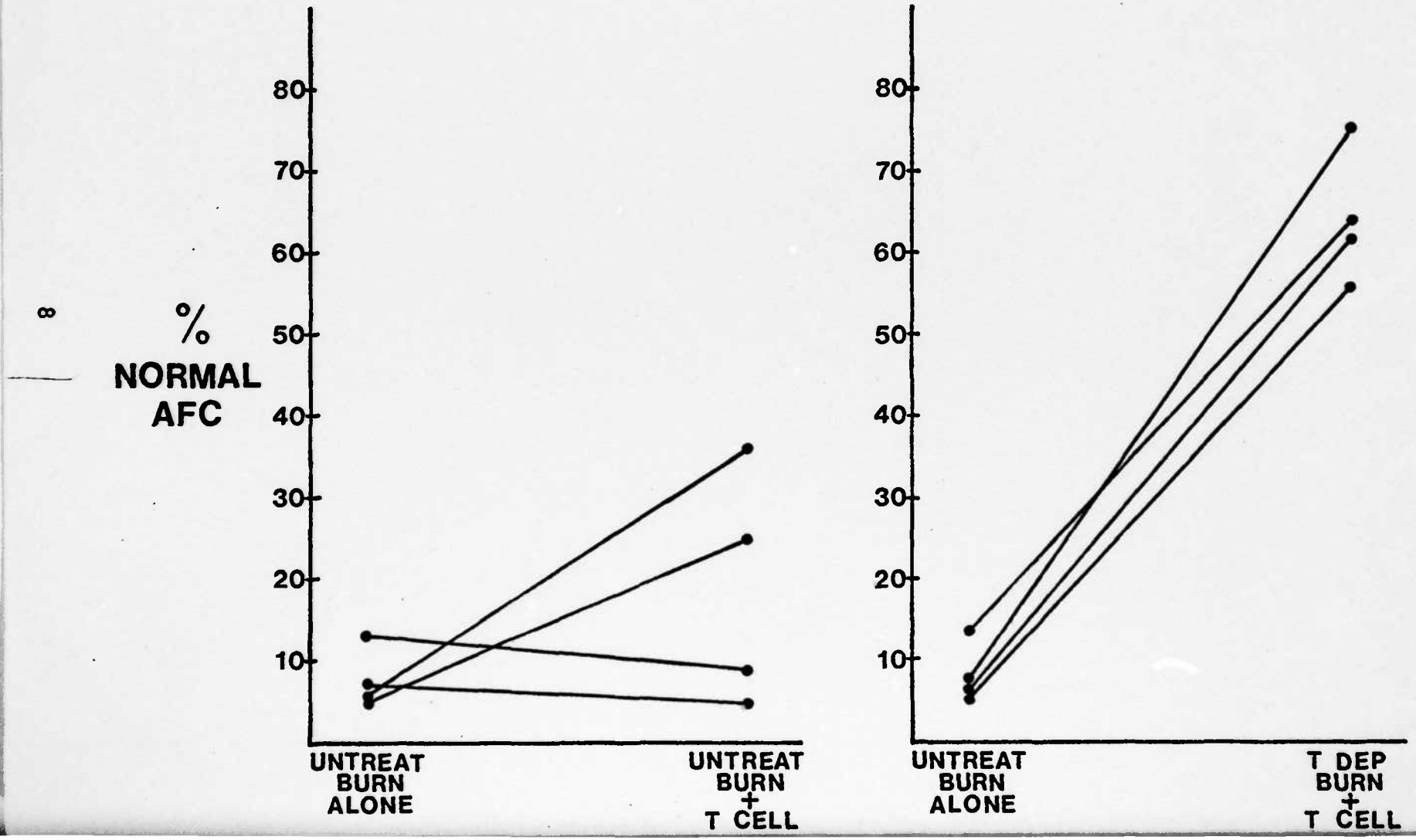


Figure
II

PRESENCE OF INCREASED T SUPPRESSOR ACTIVITY IN LEUKOCYTES FROM THERMALLY INJURED MICE



REQUIREMENT FOR ADDITIONAL LYMPHOKINS FOR TOTAL RESTORATION OF BURN INDUCED IMMUNE DEFECT

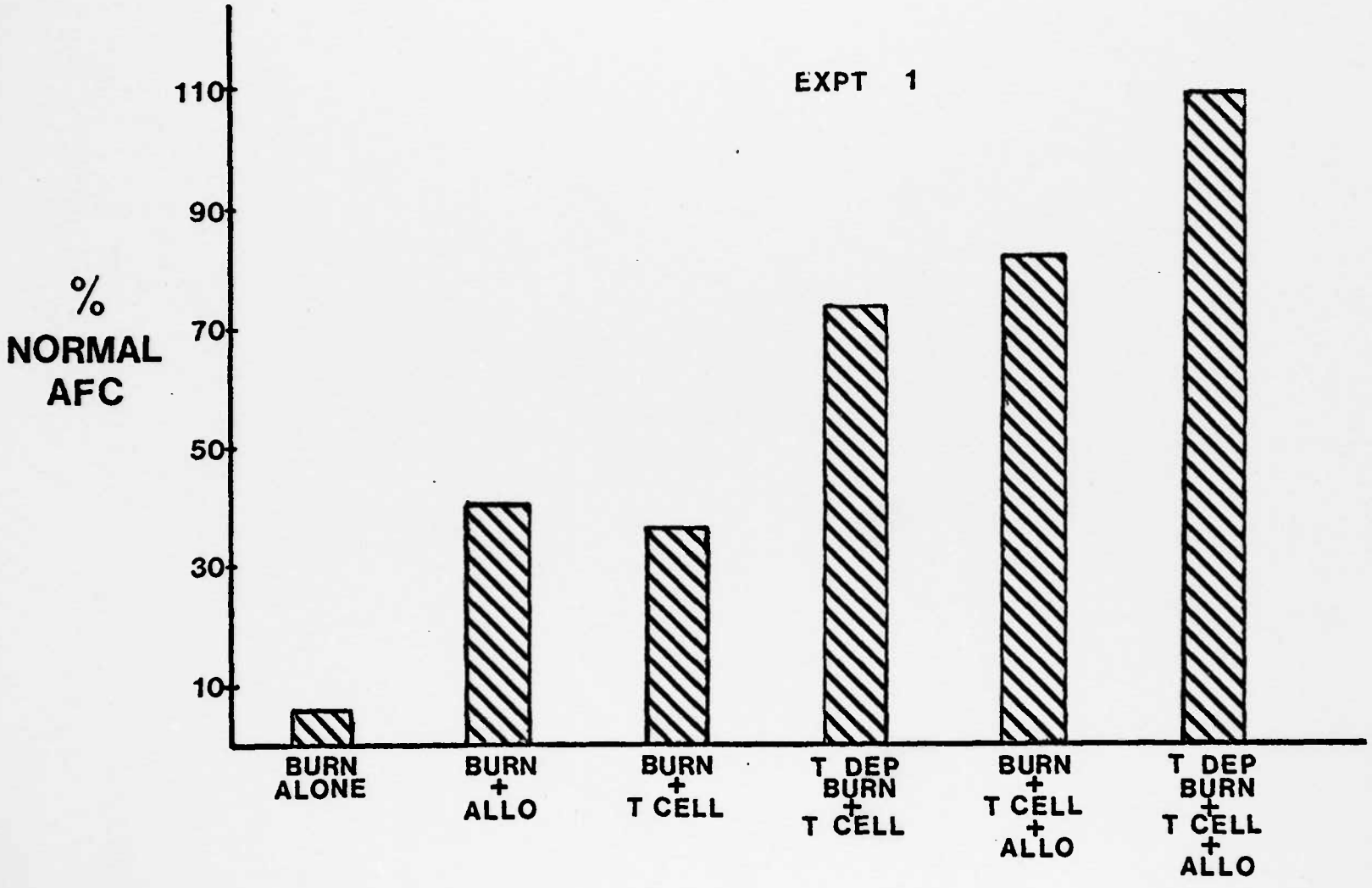


Figure
IV

REQUIREMENT FOR ADDITIONAL LYMPHOKINS FOR TOTAL RESTORATION OF BURN INDUCED IMMUNE DEFECT

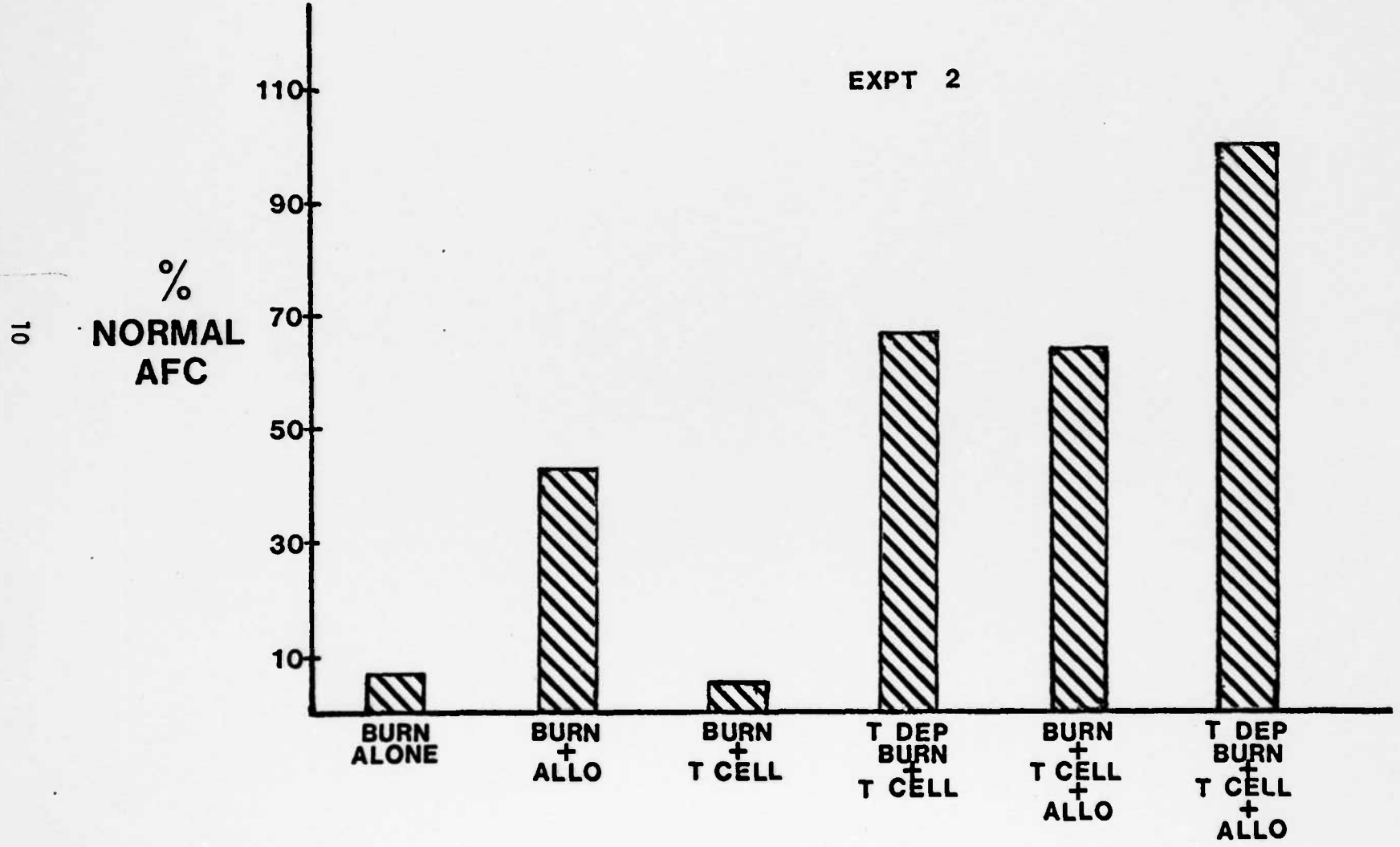


Figure
V

INDICATION OF AN A CELL DEFECT IN THERMALLY INJURED MICE

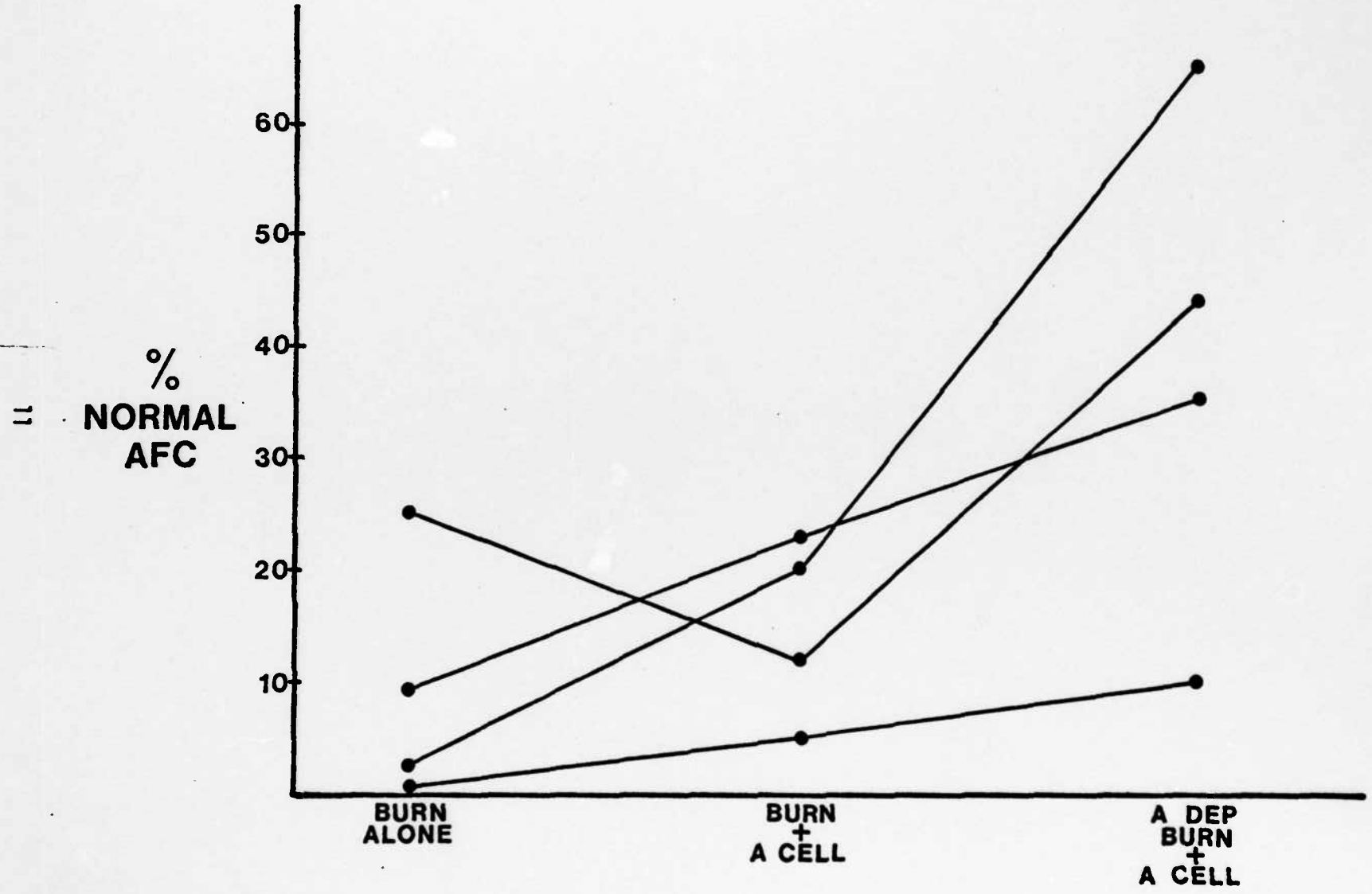


Figure VI

COMPARISON OF RESTORATION OF IMMUNE RESPONSE BY REFEEDING OR TOTAL PARENTERAL NUTRITION

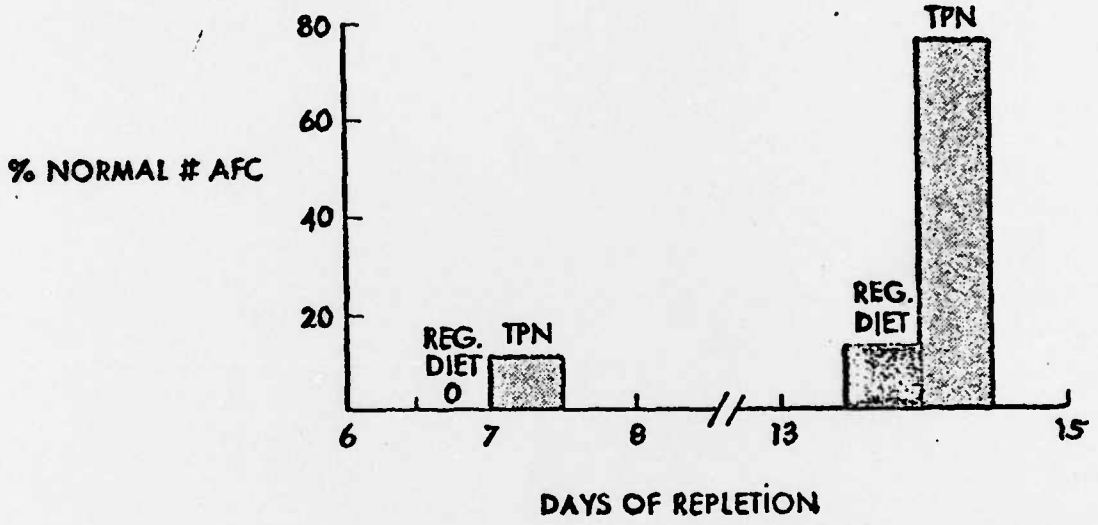


Figure VII

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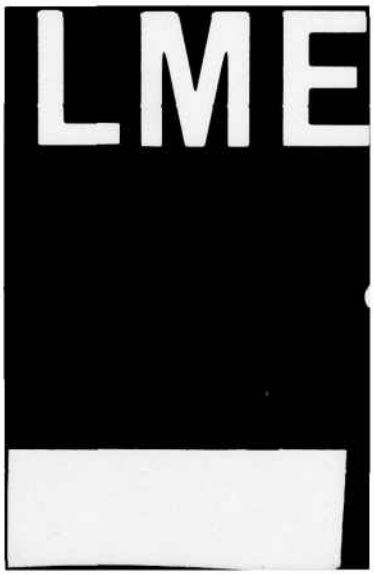
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